REMARKS

Reconsideration of the above-identified application in view of the amendment above and the remarks below is respectfully requested.

Claims 2-8, 30, and 37-39 have been canceled in this paper. Claims 1, 9-29, 31-36 and 40 have been amended in this paper. No new claims have been added in this paper. Therefore, claims 1, 9-29, 31-36 and 40 are pending and are under active consideration.

Claims 3-4 and 6-8 stand objected to under 37 CFR 1.75(c) "as being of improper dependent form for failing to further limit the subject matter of a previous claim."

Without acquiescing in the propriety of the objection, Applicant notes that claims 3-4 and 6-8 have been canceled. Therefore, the objection is moot and should be withdrawn.

Claims 1-40 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> a. Claims 1-39 are confusing because it cannot be determined what is encompassed by the term, "characterized in that." The scope of the phrase is unclear, and it is suggested to use conventional U.S. claim language, such as "comprising," or "wherein."
>
> b. Claims 1, 8-9, 11-18, 23-27, and 30 are confusing because claims 1, 8-9, 11-13, 15, 23-24, and 30 do not recite any active steps. For instance, "is amplified," is not considered a positive, active step. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. See Ex parte Erlich, 3 USPQ2d, p. 1011 (Bd. Pat. App. Int. 1986).
>
> c. Claims 1-40 are confusing because it cannot be determined what is encompassed by "target DNA," and "background DNA," first cited in claim 1, and also appears in numerous dependent claims. It is unclear from the claim and specification how one can distinguish between the two types of DNA. Furthermore, not providing such

description also renders the invention unclear, so it is suggested to provide further clarification.

d. Claim 1 recites the limitation "the 5-methylcytosine bases." There is insufficient antecedent basis for this limitation in the claim.

e. Claim 1 is confusing because it is unclear how amplifying the chemically treated DNA sample with the use of at least 2 primer oligonucleotides as well as a polymerase and nucleotide mixture leads to a preferred amplification of the target DNA over the background DNA. This is unclear partially because it is unclear how to distinguish between the target DNA and background DNA, as mentioned above, and partially because the step does not provide a clear indication of how this preferred amplification occurs.

f. Claims 3 and 6 are confusing because it cannot be determined what is encompassed by "comparatively small concentration." It is unclear what such a concentration encompasses and the specification does not provide further clarification.

g. Claim 9 is confusing because the claim recites that terminating dideoxynucleotides are additionally used, however, since claim 1 only requires "a nucleotide mixture," which encompasses dideoxynucleotides, it is unclear what the terminating dideoxynucleotides are in addition to. Clarification is required.

h. Claim 10 recites the limitation "the denaturing temperature," and "the PCR amplification," in claim 1. There is insufficient antecedent basis for this limitation in the claim.

i. Claim 15 is confusing because it cannot be determined what is encompassed by the entire claim. It is unclear what exactly the other oligonucleotide or PNA oligomer is binding to, especially within the context of binding to background DNA, since it is unclear what exactly is meant by such a term. Furthermore, the description of "t" and "a" is very confusing. For instance, the claim states that "t" represents thymine at a position which correlates with an unmethylated cytosine prior to bisulfite treatment, however, this is confusing because in claim 1, the treatment converts all cytosines to uracil. It is unclear how "'a' correlates to such a thymine position" – it is unclear what is meant by this phrase.

j. Claim 15 recites the limitation "bisulfite treatment" in claim 1. There is insufficient antecedent basis for this limitation in the claim.

k. Claim 20 recites the limitation "the other oligonucleotides" in claim 1. There is insufficient antecedent basis for this limitation in the claim.

l. Claim 25 recites the limitation "the reporter oligonucleotide" in claim 24. There is insufficient antecedent basis for this limitation in the claim.

m. Claim 26 recites the limitation "the reporter oligonucleotide or the reporter oligonucleotides" according to any one of claims 18 to 22. There is insufficient antecedent basis for these limitations in the claim.

n. Claims 37-39 provide for the use of the method according to claim 1, but, since the claim does not set forth any steps involved in the method/process, it is unclear what method/process applicant is intending to encompass. A claim is indefinite where it merely recites a use without any active, positive steps delimiting how this use is actually practiced. Although the claims recite the use of a method comprising particular steps, the claim does not actually provide any steps to how the method is used for the intended purpose, and therefore, the method/process applicant is intending to encompass is unclear.

o. Claim 40 is confusing because it is drawn to a nucleotide mixture according to claim 2, however, claim 2 is drawn to a method. Clarification is required.

Without acquiescing in the propriety of the rejection, Applicant notes that claims 2-8, 30, and 37-39 have been canceled and that claims 1, 9-29, 31-36 and 40 have been extensively amended. Applicant respectfully submits that the claims, as amended, are definite.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 30 stands rejected under 35 U.S.C. 112, first paragraph, "as failing to comply with the enablement requirement."

Without acquiescing in the propriety of the rejection, Applicant notes that claim 30 has been canceled. Therefore, the rejection is moot and should be withdrawn.

Claims 2-8 stand rejected under 35 U.S.C. 101 "because the disclosed invention is inoperative and therefore lacks utility."

Without acquiescing in the propriety of the rejection, Applicant notes that claims 2-8 have been canceled. Therefore, the rejection is moot and should be withdrawn.

Claims 37-39 stand rejected under 35 U.S.C. 101 "because the claimed recitation of a use, without setting forth any steps involved in the process, results in an improper definition of a process, i.e., results in a claim which is not a proper process claim under 35 U.S.C. 101."

Without acquiescing in the propriety of the rejection, Applicant notes that claims 37-39 have been canceled. Therefore, the rejection is moot and should be withdrawn.

Claims 1, 10-12, 14, 19-22, 26-27, 30-32 and 40 stand rejected under 35 U.S.C. 102(b) "as being anticipated by Herman et al (US 6,265,171)."

Applicant respectfully traverses the subject rejection. In the present application, a method is described in which the target DNA is preferably amplified due to its different base composition in comparison to the background DNA. It turns out that, in the analysis of differential methylation, it is not always necessary to design different primer pairs. Instead, the composition of nucleotides present in the amplification reaction can also result in amplification of the preferred type of DNA only – the target DNA. This is possible because "differentially methylated DNA", after chemical treatment , for example with bisulfite, translates into differential amounts of cytosines present in the converted DNA. A target sequence which, due to a low number of methylated cytosines, after

treatment with bisulfite, is low in cytosine, will preferably be amplified when there is rather little cytosine in the nucleotide mixture and the background sequence, which is high in cytosine content (due to high number of methylated cytosines), will not be amplified or only to a very low extent.

Claim 1, from which claims 10-12, 14, 19-22, 26-27 and 30-32 depend, has been amended herein and now recites "[a] method for the detection of cytosine methylation in DNA samples, comprising the following steps:

chemically treating a genomic DNA sample which comprises unmethylated DNA to be investigated, which is the target DNA, and methylated DNA, which is the background DNA, such that all unmethylated cytosine bases are converted to uracil, while 5-methylcytosine bases remain unchanged;

amplifying the chemically treated DNA sample with the use of at least 2 primer oligonucleotides as well as a polymerase and a nucleotide mixture, wherein said nucleotide mixture contains one of (i) 2'-deoxyguanosine triphosphate (dGTP), 2'-deoxyadenosine triphophate (dATP), 2'-deoxythymidine triphosphate (dTTP), wherein dTTP may be alternatively replaced with 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxycytidine triphosphate (dCTP), wherein the initial concentration of dCTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture, and (ii) 2'-deoxycytidine triphosphate (dCTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate (dTTP), wherein dTTP may be alternatively replaced with 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxyguanosine triphosphate (dGTP), wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture; and

concluding the methylation state in the target DNA from the presence of an amplificate or its quantity."

Support for the present amendment to claim 1 may be found in the specification, for exmaple, in original claims 4 and 7 (which were not rejected on the basis of prior art), as well as in the paragraph bridging pages 6 and 7, in the second full paragraph on page 8, and in Example 1.

Claim 1 is neither anticipated by nor rendered obvious over Herman et al. for at least the reason that Herman et al. does not teach or suggest the amplification of unmethylated DNA in the presence of a large amount of methylated background DNA by simply adjusting the nucleotide mixture composition when amplifying the chemically treated DNA, wherein said nucleotide mixture contains either (i) dGTP, dATP, dTTP (or dUTP), and dCTP, wherein the initial concentration of dCTP is at most half as much as the average initial concentration of the other three nucleotides in the nucleotide mixture or (ii) dCTP, dATP, dTTP (or dUTP), and dGTP, wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture.

Claim 40 is patentable over Herman et al. for similar reasons to those discussed above in connection with claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1, 11-12, 14 and 20-27 stand rejected under 35 U.S.C. 102(b) "as being anticipated by Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation," Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pgs. i-viii.'

Applicant respectfully traverses the subject rejection. Claim 1, from which claims 1-12, 14 and 20-27 depend, is neither anticipated by nor rendered obvious over Eads et al. for at least the reason that Eads et al. does not teach or suggest the amplification of unmethylated DNA in the presence of a large amount of methylated background DNA by simply adjusting the nucleotide mixture composition when amplifying the chemically treated DNA, wherein said nucleotide mixture contains either (i) dGTP, dATP, dTTP (or dUTP), and dCTP, wherein the initial concentration of dCTP is at most half as much as the average initial concentration of the other three nucleotides in the nucleotide mixture or (ii) dCTP, dATP, dTTP (or dUTP), and dGTP, wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 40 stands rejected under 35 U.S.C. 102(b) as being anticipated by Das et al. (US 6,143,504)."

Applicant respectfully traverses the subject rejection. Claim 40 has been amended in this paper and now recites "[a] kit consisting of a reagent containing a bisulfite, primers for the amplification and a nucleotide mixture one of (i) 2'-deoxyguanosine triphosphate (dGTP), 2'-deoxyadenosine triphophate (dATP), 2'-deoxythymidine triphosphate (dTTP) or 2'-deoxyuridine triphosphate (dUTP), and 2'-deoxycytidine triphosphate (dCTP), wherein the initial concentration of dCTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture, and (ii) 2'-deoxycytidine triphosphate (dCTP), 2'-deoxyadenosine triphosphate (dATP), 2'-deoxythymidine triphosphate (dTTP) or 2'-deoxyuridine triphosphate

(dUTP), and 2'-deoxyguanosine triphosphate (dGTP), wherein the initial concentration of dGTP is at most half as much as the average initial concentration of the other three nucleotides in said nucleotide mixture."

Claim 40 is neither anticipated by nor rendered obvious over Das et al. for at least the reason that Das et al. does not teach or suggest at least one of the specific nucleotide mixtures recited in the claim.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 40 stands rejected under 35 U.S.C. 102(b) "as being anticipated by Cottrell (US 6,960,436)."

Applicant respectfully traverses the subject rejection. Claim 40 is neither anticipated by nor rendered obvious over Cottrell for at least the reason that Cottrell does not teach or suggest at least one of the specific nucleotide mixtures recited in the claim.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 9 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation,' Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pgs. i-viii, in view of Yuanxiang et al., 'Use of a Single Sequencing Termination Reaction to Distinguish Between Cytosine and 5-Methylcytosine in Bisulfite-Modified DNA,' Biotechniques, May 1997, Vol. 22, pp. 850-853."

Applicant respectfully traverses the subject rejection. Claim 9 depends from claim 1. Claim 1 is patentable over each of Herman et al. and Eads et al. for at least the reasons discussed above. Yuanxiang et al. fails to cure all of the deficiencies of Herman et al. and Eads et al. with respect to

claim 1. Therefore, based at least on its dependency from claim 1, claim 9 is patentable over the applied combination of references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claim 13 stands rejected under 35 U.S.C. 103(a) "as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation,' Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pgs. i-viii, in view of Guetig (US 2004/0248120)."

Applicant respectfully traverses the subject rejection. Claim 13 depends ultimately from claim 1. Claim 1 is patentable over each of Herman et al. and Eads et al. for at least the reasons discussed above. Guetig fails to cure all of the deficiencies of Herman et al. and Eads et al. with respect to claim 1. Therefore, based at least on its dependency from claim 1, claim 13 is patentable over the applied combination of references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 15-18 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over either one of Herman et al (US 6,265,171) or Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation,' Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pgs. i-viii, in view of Orum et al., 'Single base pair mutation analysis by PNA directed PCR clamping,' Nuc. Acids Res., 1993, Vol. 21, No. 23, pp. 5332-5336."

Applicant respectfully traverses the subject rejection. Claims 15-18 depend ultimately from claim 1. Claim 1 is patentable over each of Herman et al. and Eads et al. for at least the reasons discussed above. Orum et al. fails to cure all of the deficiencies of Herman et al. and Eads et al. with

respect to claim 1. Therefore, based at least on their respective dependencies from claim 1, claims 15-18 are patentable over the applied combination of references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 28 and 29 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation,' Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pg. i-viii."

Applicant respectfully traverses the subject rejection. Claims 28 and 29 depend from claim 1. Claim 1 is patentable over Eads et al. for at least the reasons discussed above. Therefore, based at least on their respective dependencies from claim 1, claims 28 and 29 are patentable over Eads et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 28 and 29 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Herman et al (US 6,265,171) in view of Eads et al., 'MethyLight: a high-throughput assay to measure DNA methylation,' Nucleic Acids Research, 2000, Vol. 28, No. 8, e32, pg. i-viii."

Applicant respectfully traverses the subject rejection. Claims 28 and 29 depend from claim 1. Claim 1 is patentable over Herman et al. and Eads et al., whether viewed individually or in combination, for at least the reasons discussed above. Therefore, based at least on their respective dependencies from claim 1, claims 28 and 29 are patentable over Herman et al. in view of Eads et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 33-36 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Herman et al (US 6,265,171) in view of Olek et al. (WO 01/77378 A2, published October 18, 2001)."

Applicant respectfully traverses the subject rejection. Claims 33-36 depend ultimately from claim 1. Claim 1 is patentable over each of Herman et al. for at least the reasons discussed above. Olek et al. fails to cure all of the deficiencies of Herman et al. with respect to claim 1. Therefore, based at least on their respective dependencies from claim 1, claims 33-36 are patentable over the applied combination of references.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1 and 8-36 stand rejected "on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-36 of U.S. Patent No. 7,229,759."

Insofar as the subject rejection relates to claims 8 and 30, the rejection is moot in view of Applicant's cancellation of claims 8 and 30 in this paper. Insofar as the subject rejection relates to claims 9-29 and 31-36, Applicant respectfully traverses the subject rejection. As noted above, claim 1, from which claims 9-29 and 31-36 depend, has been amended in this paper. Thus amended, claim 1 now recites, amongst other things, particular nucleotides mixtures. These nucleotide mixtures patentably distinguish claim 1 over claims 1-36 of U.S. Patent No. 7,229,759.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on May 21, 2009

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